Amendments to the Specification:

Please amend the application as follows

The paragraph [0004] beginning on page 1 has been amended as follows:

--**[0004]** In the airways, mucin proteins form a protective barrier on the airway epithelial cells, and interact with cilia to trap and clear pathogens (*e.g.*, microorganisms), particulate matter, irritants and pollutants (*e.g.*, tobacco smoke and ~~sulphur~~ sulfur dioxide). Mucus secretions in the airway are produced from two different secretory cell populations, the surface epithelial goblet cells and the mucous cells in the submucosal glands. At least eight mucin genes are expressed (at the mRNA level) in the upper and lower respiratory tracts. Of these, only the *MUC5AC* and *MUC5B* polypeptides have been conclusively demonstrated to be major components of human airway secretions (Hovenberg *et al.*, *Biochem. J.*, 318(Pt. 1, Vol. 17):319-324 [1996]; Hovenberg *et al.*, *Glycoconjugate Jour.*, 13(5):839-847 [1996]; Thornton *et al.*, *J. Biol. Chem.*, 272(14):9561-9566 [1997]; and Wickström *et al.*, *Biochem. Jour.*, 334(Pt. 3, Vol. 14):685-693 [1998]). *MUC5B* is also expressed in other tissues, including, for example, pancreas and gall bladder.--

The paragraph [0027] beginning on page 8 has been amended as follows:

--**[0027]** FIGS. 3A-3D show light microscopy images of *in situ* nucleic acid hybridizations of human bronchial tissue cross sections from patients with UIP or emphysema. FIG. 3A shows a section of the trachea tissue of a UIP patient after the *in situ* hybridization. A 48-mer oligonucleotide (SEQ ID NO: 1) corresponding to the antisense sequence of the human *MUC5B* tandem repeats region was used as the *in situ* probe. Original magnification was 100X. FIG. 3B shows a cross section of surface epithelium of the bronchiole region of the UIP ~~paitent's~~ patient's lung. A *MUC5B* oligonucleotide as described in FIG. 3A was used as the *in situ* probe. Original magnification was 400X. FIG. 3C shows an *in situ* hybridization in a human tracheal tissue section derived from a patient with emphysema. A *MUC5B* oligonucleotide as described in FIG. 3A was used as the *in situ* probe. Original magnification was 100X.

FIG. 3D shows an *in situ* hybridization in a human tracheal tissue section derived from a patient with emphysema using a *MUC5AC* nucleic acid probe (SEQ ID NO: 2). Original magnification was 100X.--

The paragraph [0028] beginning on page 8 has been amended as follows:

--**[0028]** FIGS. 4A-4B show Northern blot analyses of *MUC5B* message expression in various human cell cultures. The top portions of these blots are probed using a 48 basepair ^{32}P-end labeled nucleic acid probe derived from the repetitive repeat region of the human *MUC5B* gene. FIG. 4A, top panel, shows Northern blot analysis of total RNA isolated from primary explant human tracheobronchial epithelial (TBE) cell cultures. These cultures were maintained under four different culture conditions, which were standard tissue culture dishes (TC), collagen gel coated dishes (CG), Transwell™ chambers (BI), or collagen-gel coated Transwell™ chambers (BICG). Cultures were grown either in the presence (+RA) or absence (-RA) of retinoic acid at a concentration of 30 nM. FIG. 4B, top panel, shows a Northern blot using total RNA isolated from airway cultures and probed for *MUC*5B message ~~expession~~ expression. Cells used in the analysis were primary TBE cells, HBE1 cells and BEAS-2B (S clone) cells. The cells used in FIG. 4B were plated using BICG culture conditions contained 30 nM retinoic acid. Following analysis with the *MUC5B* probe, the blots used in FIGS. 4A and 4B were stripped and reprobed with an 18S rRNA cDNA probe as a reference for RNA loading normalization.--

The paragraph [0030] beginning on page 9 has been amended as follows:

--**[0030]** FIGS. 6A-6H show 22,773 basepairs of human *MUC5B* genomic region isolated and sequenced from the Cos-1 genomic cosmid clone (SEQ ID NO: 6). This 22.7 kB encompasses 4169 basepairs of sequence upstream of the transcription start site, the 5'-UT and the 30 exons/introns upstream of the *MUC5B* large central exon.--

The paragraph [0032] beginning on page 9 has been amended as follows:

--**[0032]** ~~FIG.8~~ FIG. 8 shows the nucleotide sequence of the *MUC5B* gene 5'-UTR, adjacent promoter proximal flanking region and the first exon (SEQ ID NO: 35). Only 2007 basepairs of the sequenced 22,773 basepairs are shown. Various putative DNA motifs are underlined. The transcription start site is indicated by an arrow. The predicted first exon coding region is underlined, and the corresponding predicted signal peptide amino acid sequence is shown using standard letter codes (SEQ ID NO: 36).--

The paragraph [0034] beginning on page 10 has been amended as follows:

--**[0034]** FIG. 10 shows the *MUC5B* genomic nucleotide sequence encompassing positions -1098 through +7 that were subcloned into the MUC5B-b1 luciferase reporter construct (SEQ ID NO: 31).--

The paragraph [0035] beginning on page 10 has been amended as follows:

--**[0035]** FIG. 11A and 11B show the *MUC5B* genomic nucleotide sequence encompassing positions -4169 through +7 that were subcloned into the MUC5B-b2 luciferase reporter construct (SEQ ID NO: 32).--

The paragraph [0036] beginning on page 10 has been amended as follows:

--**[0036]** FIG. 12 shows the *MUC5B* genomic nucleotide sequence encompassing positions -13 through +2738 that were subcloned into the MUC5B-il luciferase reporter construct (SEQ ID NO: 33).--

The paragraph [0059] beginning on page 18 has been amended as follows:

--**[0059]** As used herein, the terms "nucleic acid molecule encoding," "DNA sequence encoding," and "DNA encoding" and similar phrases refer to the order or sequence of deoxyribonucleotides along a strand of deoxyribonucleic acid encoding a particular polypeptide. The order of the d~~eoxynbonucleotides~~ deoxyribonucleotides determines the order of the amino acids in the polypeptide chain. The DNA sequence thus codes for the amino acid sequence.--

The paragraph [0062] beginning on page 19 has been amended as follows:

--**[0062]** As used herein, the term "vector" is used in reference to nucleic acid molecules that ~~cam~~ can be used to transfer DNA segment(s) from one cell to another. The terms "vehicle" or "construct" or "plasmid" are sometimes used interchangeably with "vector." In some embodiments, a vector "backbone" comprises those parts of the vector which mediate its maintenance and enable its intended use (*e.g.,* the vector backbone contains sequences necessary for replication, genes imparting drug or antibiotic resistance, a multiple cloning site, and possibly operably linked promoter/enhancer elements which enable the expression of a cloned nucleic acid). The cloned nucleic acid (*e.g.,* such as a cDNA coding sequence, or an amplified PCR product) is inserted into the vector backbone using common molecular biology techniques. Vectors are often derived from plasmids, bacteriophages, or plant or animal viruses. A "cloning vector" or "shuttle vector" or "subcloning vector" contain operably linked parts which facilitate subcloning steps (*e.g.,* a multiple cloning site containing multiple restriction endonuclease sites). A "recombinant vector" indicates that the nucleotide sequence or arrangement of its parts is not a native configuration, and has been manipulated by molecular biological techniques. The term implies that the vector is comprised of segments of DNA that have been artificially joined. A "reporter construct" is a vector encoding a suitable "reporter" gene. The transcription of the reporter gene is typically regulated by heterologous promoter sequences.--

The paragraph [0070] beginning on page 21 has been amended as follows:

--**[0070]** As used herein, the term "established" or "established culture" is a cell culture, most typically a mammalian cell culture, that has acquired the ability to grow ~~indefinately~~ indefinitely in culture (in contrast to a primary cell culture). An established cell culture may or may not display traits of transformed cells. Mammalian cells can be established artificially, *e.g.*, by the stable forced expression of the SV-40 large T-antigen.--

The paragraph [0076] beginning on page 23 has been amended as follows:

--[0076] As used herein, the term *"in vitro"* refers to an artificial ~~enviromnent~~ environment and to processes or reactions that occur within an artificial environment. The term "*in vivo*" refers to the natural environment (*e.g.,* in an animal or in a cell) and to processes or reactions that occur within a natural environment. The definition of an *in vitro* versus *in vivo* system is particular for the system under study.--

The paragraph [0087] beginning on page 26 has been amended as follows:

--[0087] The term "promoter activity" when made in reference to a nucleic acid sequence refers to the ability of the nucleic acid sequence to initiate transcription of a downstream deoxyribonucleic acid (DNA) sequence into a ribonucleic acid (*i.e.*, RNA) sequence (*e.g.*, messenger-RNA, transfer-RNA or ~~ribhosomal~~ ribosomal-RNA).--

The paragraph [0091] beginning on page 27 has been amended as follows:

--[0091] The term "transgenic" is used herein as an adjective to describe the property, for example, of an animal or a construct, of harboring a transgene. For instance, as used herein, a "transgenic organism" is any animal, preferably a non-human mammal, in which one or more of the cells of the animal contain heterologous nucleic acid introduced by way of human intervention, such as by ~~trangenic~~ transgenic techniques well known in the art. The nucleic acid is introduced into the cell, directly or indirectly by introduction into a precursor of the cell, by way of deliberate genetic manipulation, such as by microinjection or by infection with a recombinant virus. The term genetic manipulation does not include classical cross-breeding, or *in vitro* fertilization, but rather is directed to the introduction of a recombinant DNA molecule. This molecule may be integrated within a chromosome, or it may be extrachromosomally replicating DNA. In the transgenic animals described herein, the transgene is in the form of a ~~reporte~~ reporter gene, the transcription of which is driven by MUC5B promoter sequences (*e.g.*, SEQ ID NOs: 31 or 32). The terms "founder line" and "founder animal" refer to those animals that are the mature product of the embryos

to which the transgene was added, *i.e.*, those animals that grew from the embryos into which DNA was inserted, and that were implanted into one or more surrogate hosts.--

The paragraph [0095] beginning on page 29 has been amended as follows:

--**[0095]** For the purpose of studying *MUC5B* transcriptional regulation and genomic structure, genomic DNA encompassing the *MUC5B* transcriptional start site was isolated. To isolate genomic DNA clones containing *MUC5B* nucleotide sequence, an initial low-~~stingency~~ stringency hybridization strategy using a *MUC2* amino-terminal and promoter proximal region nucleic acid probe was used to screen a Clontech human genomic library (the *MUC2* and *MUC5B* genes contain strong homology in their promoter and amino-terminal domains). This initial screening of 10^6 cosmid clones identified eight (8) candidate clones, which were then subjected to a secondary screening using *MUC5AC* cDNA sequences as a Southern blot probe under high stringency conditions. This secondary screen of the initial eight positive clones yielded only a single positive cosmid clone, which was termed Cos-1. The detailed methodology and reaction conditions used in this isolation are provided in EXAMPLE 4.--

The paragraph [0098] beginning on page 30 has been amended as follows:

--[0098] Another depiction of part of the 22.7 kB sequence proximal to the transcription start site showing predicted landmarks of the gene is shown in FIG. 8 (SEQ ID NO: 35). This Figure shows the predicted *MUC5B* transcription start site, a TATA box 30 nucleotides upstream of the transcription start site and a putative translation start codon ATG embedded within a Kozak consensus sequence. Furthermore, based on the deduced amino acid sequence, the extreme amino-terminal coding region contained a classic putative secretory signal sequence . This feature is consistent with the secretory nature of the mucin gene products in the airway and various other organs. Several putative motifs for various transcription factor binding

sites were also identified upstream of the transcription start site, as indicated in FIG. 8 (SEQ ID NO: 35).--

The paragraph [0106] beginning on page 32 has been amended as follows:

--[0106] After tailing, the resulting double stranded cDNA products were used in polymerase chain reactions (PCR) with nested primers within the 3'-end and the 5'-anchor oligo d(T) adapter. PCR amplification was carried out using various primer combinations (*see*, TABLE 2). The resulting PCR products were subcloned into the TA Cloning® vector (Invitrogen, Carlsbad, CA) and sequenced. Since there should be only one common DNA sequence adjacent to oligo d(T) and oligo d(A) adapters, this DNA sequence should be identical to that of the 5'-end message upstream to the +250/+230 primer. A major advantage of this approach is the use of PCR, which allows the amplification of the 5'-ends of low abundance messages. The sequence analysis of the PCR products generated above identified a transcription start site located ~~located~~ at approximately basepair position 4176, as shown in FIG. 6, and GenBank Accession No. AF107890 (*and see*, FIG. 8). This position is in agreement with the primer extension analysis described in EXAMPLE 5. Both approaches yielded the same conclusion, suggesting that the transcription start site is 18604 basepairs upstream of the large central exon (using the numbering convention of FIG. 8). This putative transcription start site is different from the sites previously reported (Offner *et al.*, *Biochem. Biophys. Res. Comm.*, 251(1):350-355 [1998]; and Van Seuningen *et al.*, *Biochemical Jour.*, 348 Pt 3(12):675-686 [2000]).--

The paragraph [0107] beginning on page 33 has been amended as follows:

--[0107] In order to study the transcriptional regulation of the *MUC5B* gene, and also to define minimal promoter elements controlling *MUB5B* ~~tanscription~~ transcription in response to environmental conditions, luciferase reporter constructs under the transcriptional control of *MUC5B* gene sequences were constructed, as

described in EXAMPLE 7. The gene sequences used to make these reporter constructs were derived from the isolated genomic DNA described in EXAMPLE 4.--

The paragraph [0132] beginning on page 40 has been amended as follows:

--[0132] Pseudopregnant, foster or surrogate mothers are prepared for the purpose of implanting embryos, which have been modified by introducing the transgene. Such foster mothers are typically prepared by mating with vasectomized males of the same species. The stage of the pseudopregnant foster mother is important for successful implantation, and it is species dependent. For mice, this stage is about 2-3 days pseudopregnant. Recipient females are mated at the same time as donor females. Although the following description relates to mice, it can be ~~adepted~~ adapted for any other non-human mammal by those skilled in the art. At the time of embryo transfer, the recipient females are anesthetized with an intraperitoneal injection of 0.015 ml of 2.5% avertin per gram of body weight. The oviducts are exposed by a single midline dorsal incision. An incision is then made through the body wall directly over the oviduct. The ovarian bursa is then torn with watchmaker's forceps. Embryos to be transferred are placed in DPBS (Dulbecco's phosphate buffered saline) and in the tip of a transfer pipet (about 10 to 12 embryos). The pipet tip is inserted into the infundibulum and the embryos transferred. After the transfer, the incision is closed by two sutures. The number of embryos implanted into a particular host will vary by species, but will usually be comparable to the number of off spring the species naturally produces.--

The paragraph [0137] beginning on page 42 has been amended as follows:

--[0137] The animals of this invention can be used as a source of cells, differentiated or precursor, which can be immortalized in cell culture if desired. Cells containing a MUC5B-reporter can be isolated from the ~~trangenic~~ transgenic animal and established *in vitro* as cell lines and used for drug screening. Thus, the transgenic animals of this invention can be used as a source of cells for cell culture. Tissues of

transgenic mice are analyzed for the presence and/or expression of the MUC5B-reporter transgene as described, and cells or tissues carrying the reporter transgene are cultured, using standard tissue culture techniques (*see*, EXAMPLE 10).--

The paragraph [0164] beginning on page 51 has been amended as follows:

--[0164] Tissue samples from the patients listed in TABLE 1 were processed for airway epithelial cell isolation and subsequent culture using techniques known in the art. For example, this ~~procedue~~ procedure is described in Wu *et al.*, *European Respiratory Journal* 10(10):2398-2403 [1997] and Robinson and Wu., *J. Tiss. Cult. Meth.*, 13:95-102 [1991]). Briefly, human surgical or necropsy specimens were obtained and immersed in minimum essential medium (MEM; GIBCO Laboratories) with L-glutamine and without sodium pyruvate or sodium bicarbonate. The specimens were rinsed in this same medium 2 to 5 times, then immersed in a dissociation solution comprising trypsin protease and EDTA overnight at 4˚C. The next day, the mucosal surface was washed multiple times with ice-cold MEM with 10% fetal bovine serum. The washes were pooled and centrifuged to isolate the suspended cells.--

The paragraph [0165] beginning on page 51 has been amended as follows:

--[0165] The primary tracheobronchial epithelial (TBE) cells contained in the cell pellet were resuspended in a growth medium and cultured in conditions to stimulate a mucoid/ciliary differentiation pathway. This complete serum-free growth medium comprised F-12 or DME/F12 (1:1) media (GIBCO Laboratories) supplemented with insulin (5 µg/ml), transferrin (5 µg/ml), epidermal growth factor (EGF; 10 ng/ml), dexamethasone (DEX; 0.1 µM), cholera toxin (20 ng/ml), bovine hypothalamus extract (BHE; 15 µg/ml), all-trans-retinoic acid (RA; 30 nM) and ~~calcuim~~ calcium chloride. The medium was changed the following day, and every other day thereafter. The cells were initially innoculated in plastic tissue culture dishes for propagation, and subjected to serial cultivation and passaging as necessary. In general, the primary human TBE cells

maintained on plastic culture surfaces were passaged from 1 to 5 times with a total of 20 to 25 population doublings.--

The paragraph [0182] beginning on page 56 has been amended as follows:

--**[0182]** To further elucidate ~~poatterns~~ patterns of *MUC5B* gene regulation, the expression patterns of *MUC5B* in primary and established cultures of TBE-derived human cells were studied. This example describes the isolation of RNA and the analysis of *MUC5B* gene expression using Northern blotting techniques. This example analyzes *MUC5B* gene expression in various cultured cell lines derived from airway tissues, and also under various culture conditions.--

The paragraph [0192] beginning on page 59 has been amended as follows:

--**[0192]** In view of the amino acid conservation in the 5' end (*i.e.*, amino-terminus) ~~cystine~~ cysteine-rich domains between *MUC2* and *MUC5B*, it was contemplated that this approach would identify genomic clones containing the amino-terminal and promoter region of the human *MUC5B* gene. A total of 10^6 cosmid clones were screened, of which eight were positive for hybridization to the *MUC2* probe.--

The paragraph [0199] beginning on page 61 has been amended as follows:

--**[0199]** *Restriction Mapping of the MUC5B Cosmid* - Genomic DNA from the Cos-1 cosmid was prepared and digested with *Kpn*I and *Eco*RI restriction enzymes. Southern blotting hybridization was carried out to determine which DNA fragments contain *MUC5AC* gene sequences or *MUC5B* cDNA sequences. The probe corresponding to the 3' end of the *MUC5AC* message is provided in SEQ ID NO: 5 (corresponding to nucleotide positions 1,441 through 3,108 of GenBank Accession Number Z48314). The probe corresponding to the 5' end of *MUC5B* large central exon is provided in SEQ ID NO: ~~XX~~ 34 (corresponding to nucleotide positions 1 through 809 of GenBank Accession Number Z72496).--

The paragraph [0222] beginning on page 68 has been amended as follows:

--**[0222]** The β-galactosidase reporter gene activity was assayed according to methods known in the art. Briefly, the luciferase cell extracts described above were mixed with an equal volume of β-galactosidase assay buffer (120 mM Na_2HPO_4, 80 mM NaH_2PO_4, 2 mM $MgCl_2$, 100 mM β-mercaptoethanol, 1.33 mg/ml o-nitrophenyl-beta-D-galactopyranoside [ONPG]), then read in a microplate reader (Molecular Devices) at ~~wavelenghth~~ wavelength 420 nm.--

The paragraph [0226] beginning on page 69 has been amended as follows:

--**[0226]** FIG. 13 shows the results of a transfection assay using the chimeric reporter constructs shown in FIG. 9 and passage-1 primary TBE cells. The TBE cells were also co-transfected with a *β*-galactosidase expression vector, and luciferase activity was normalized against *β*-galactosidase activity to take into account transfection efficiency variability. Relative activities of each of the reporter constructs following transfection in the TBE cells is shown, and activity is expressed as ~~as~~ units of luciferase activity per unit of *β*-gal activity (units/beta-gal). As can be seen in this FIG. 13, the reporter gene activity in MUC5B-b1 and MUC5B-b2 transfected cells was two- to five-fold higher, respectively, than those transfected with the promoterless control construct, pGL-3 (labeled "control"). However, no significant activity was observed in the transfection using the MUC5B-il construct. These results indicate that the regions -1098 to +7 and –4169 to +7 both have promoter activity, and the –4169 to +7 region contains stronger promoter activity than does the -1098 to +7 region. Furthermore, the –13 to +2738 region contained no detectable promoter activity under these conditions.--

The paragraph [0236] beginning on page 72 has been amended as follows:

--**[0236]** This example describes the analysis of *MUC5B* promoter reporter constructs carried as integrated transgenes in mice. The construction of these mice is described in EXAMPLE 9. The ~~expession~~ expression of these reporter genes is

analyzed using two different protocols (*i.e.*, one for luciferase activity analysis, and one for GFP analysis). Furthermore, the activity of these reporters is studied in response to various cytokines and environmental factors, such as interleukin-6 (IL-6), IL-17 and tobacco smoke.--